行政院國家科學委員會補助專題研究計畫成果報告

奇異變形桿菌致病因子受 *rsmA* 及 PNPG 調控之研究

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行政院國家科學委員會
專題研究計畫成果報告

一、中文摘要

Proteus mirabilis 為尿道感染的重要病原菌，此菌可以產生多種致病因子，此外 *P mirabilis* 會展現表面移行 (swarming) 的多細胞行為，此行為和 *P. mirabilis* 常上行到腎臟造成嚴重感染有密切關. *rsmA* 基因已被發現會抑制多種致病因子的表現，同時也和調控多細胞行為之 quorum sensing 系統有關. *rsmA* 相似物普遍存在於許多菌屬中，所以我們認為 *rsmA* 是一個相當重要的調控因子，而且可能是透過 quorum sensing 系統來調控許多多細胞行為的表現. p-nitrophenylglyecrol (PNPG)長久以來被用來抑制 *P. mirabilis* 的 swarming 以利於臨床分離受 *P. mirabilis* 污染的細菌，但其作用機轉不明. 我們成功選殖了 *P. mirabilis rsmA,* 發現它可以抑制表面移行，以及致病因子的表現。同時發現此基因和 *E. coli csrA, S. marcescens* 及 *E. c.c.*的 *rsmA* 相似物有相同的功能。由北方墨點實驗得知 *P. mirabilis rsmA* 抑制溶血素表現的原因乃由促其 RNA 的分解來達成。此外也發現 PNPG 會抑制表面移行分化及致病力（蛋白脢、溶血素、尿素脢、鞭毛蛋白等的表現及入侵尿道上皮細胞的能力）。可見 PNPG 有潛力被發展成對抗 *P. mirabilis* 感染的抗菌劑。

關鍵詞：*P. mirabilis,* PNPG, *rsmA,* 表面移行,致病因子

Abstract

Proteus mirabilis is an important uropathogenic bacterium. A number of virulence factors have been reported including hemolysin, protease, urease, flagella. The bacterium also exhibits a multicellular behaviour called swarming, which is involved in the ascending urinary tract infections. Recently, a global repressor called *rsmA*, which can suppress expressions of virulence factors and the synthesis of quorum-sensing signal molecules, has been reported. The wide presence of the *rsmA* homologue in many enterobacteria suggests that rsmA is one of the important regulatory genes of enterobacterial species and its function is through quorum-sensing system to modulate the expression of various phenotypic traits. In order to investigate the role of RsmA in *P. mirabilis*, we cloned the *rsmA* gene (hereafter $rsmA_{Pm}$) from *P. mirabilis*. A low-copy plasmid carrying $rsmA_{Pm}$ expressed from its native promoter in *P. mirabilis* caused suppression of swarming motility and the expression of virulence factors. RNA stability asssay indicated that $RsmA_{Pm}$ acts through promoting mRNA degradation of the virulence gene. We also observed that *rsmA* of both *Serratia marcescens* and *Erwinia carotovora* subsp. *carotovora* has similar effect on swarming motility and expression of virulence factors in *P. mirabilis*. Complementation of *E. coli csrA* mutant with $rsmA_{Pm}$ further confirmed the functional similarity between *csrA* and *rsmA*. PNPG, an anti-swarming agent, is long used for the isolation of pathogenic bacteria from specimens contaminated with swarming strains of Proteus spp, but the underlying mechanism is unclear. In order to investigate the effects of p-nitrophenylglycerol (PNPG), a potent anti-swarming agent, on the various swarming-associated traits of *P. mirabilis* and to elucidate the relationships among them, *P. mirabilis* growth rate, swarming/swimming activity, cell invasion ability, and the ability to express various virulence factors was monitored in the presence or absence of PNPG. It was found that PNPG could inhibit the growth rate, swarming differentiation, and swarming/swimming activities of *P. mirabilis.* The expression of virulence factors, such as protease, urease, haemolysin and flagellin, in *P. mirabilis* was also inhibited by PNPG. The ability of *P. mirabilis* to invade human urothelial cells was reduced dramatically in the presence of PNPG. These results suggest that PNPG has

the potential to be developed as an agent active against the effects of *P. mirabilis* infection.

Keywords: *Proteus mirabilis,* virulence factors, *rsmA*, PNPG

二、緣由與目的

Proteus mirabilis is a pathogenic gram-negative bacterium frequently causing serious urinary tract infections (UTI). The bacterium has a number of virulence factors, including hemolysin, urease, protease, flagella, and determinants that facilitate invasion of mammalian cells. In addition, *P. mirabilis* exhibits a form of multicellular behaviour termed swarming, which involved cyclical differentiation of typical vegetative cells into filamentous, ultinucleate hyperflagellate swarm cells capable of rapid and co-ordinated population migration across surface (1). The ability of *P. mirabilis* to swarm plays a role in ascending UTI, is strengthened by the discovery that differentiation of cells to hyperflagellated swarming form is coupled to the overexpression of virulence genes (2-3). A mode of gene regulation called quorum-sensing system has been reported in many bacteria involved in the control of various phenotypes (4-6). In the mode of quorum sensing system, all the bacteria will not behave such interesting phenotypes only after they reached a certain population density. The quorum- sensing signal was analyzed to be a kind of homoserine lactone (HSL), which was synthesized by Lux I (7). Chatterjee et al., in 1995 identified a global repressor gene, *rsmA* (repressor of secondary metabolites), in *E. carotovora* subsp. *carotovora* that controls the expression of extracellular enzymes , HSL synthesis, and pathogenicity (8). *E. carotovora subsp. carotovora* carrying *rsmA* showed decreased virulence factor expression and HSL signaling molecule expression (9). It is very interesting that high percentage of amino acid sequence homology was found between RsmA and *E. coli* regulatory protein CsrA (carbon storage regulator) (10). The regulatory mechanism of CsrA controlling glycogen production was proved to be via affecting *glgC* (one of the glycogen synthesis genes, encodes ADP-glucose pyrophosphorylase) mRNA stability (11). The anti-swarming agent p-nitrophenylglycerol (PNPG) has long been found invaluable for the recognition and isolation of pathogenic bacteria from specimens contaminated with swarming strains of *Proteus* spp. However, the underlying inhibitory mechanism was still unknown. In this study, we want to elucidate the PNPG and *rsmA* on the effect of swarming and virulence expression in *P. mirabilis.*

三. 結果與討論、

1. We cloned *rsmA* from *P. mirabilis*. Our data established that *P. mirabilis rsmA* is a homologue of *E. coli csrA* and $rsmA_{Ecc}$. First, the DNA sequence and the predicted products of these genes share a very high percentage of identity. Second, they all bear a putative RNA binding motif. Third, $rsmA_{Ecc}$ and $rsmA_{Sm}$ can trans-suppress swarming motility and virulence expression in *P. mirabilis* as $rsmA_{Pm}$ can. Fourth, *P. mirabilis rsmA* can complement the glycogen-excess phenotype of *E. coli csrA* mutant. Furthermore, complementation of *csrA* mutant with $rsmA_{Pm}$ also restore the wild-type cell length and abolish the biofilm formation of the *csrA* mutant.
2. Like CsrA and $RsmA_{Ecc}$, $RsmA_{Pm}$ also affect mRNA stability and consequently the cognate phenotypes.
3. It was found that PNPG could inhibit the growth rate, swarming differentiation, and swarming/swimming activities of *P. mirabilis.* The expression of virulence factors, such as protease, urease, haemolysin and flagellin, in *P. mirabilis* was also inhibited by PNPG. The ability of *P. mirabilis* to invade human urothelial cells was reduced dramatically in the presence of PNPG. These results suggest that PNPG has the potential to be developed as an agent active against the

effects of *P. mirabilis* infection.

四. 計畫成果自評

We have elucidated the effect of *rsmA* and PNPG on the swarming and virulence expression. We are now using transposon-mutagenesis to investigate the inhibitory mechanism of PNPG. We also plan to find the possible quorum-sensing signals and their genes responsible for their synthesis by different quorum-sensing detector systems.

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